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ESTIMATION OF SERUM COPPER AND ZINC LEVEL IN PATIENTS WITH ORAL SUB MUCOUS FIBROSIS AND ORAL SQUAMOUS CELL CARCINOMA

Rajeev Aggarwal,[1*]Abhiney Puri[2], Rajat Nangia[3], Nitish Bhat[4]

[1]PG Student, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India
[2]Prof and Head, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India
[3]Reader, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India
[4]Sr. Lecturer, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India

ABSTRACT

Trace elements are receiving too much attention as they are found to be significantly altered in head and neck, lung and breast carcinomas, and there is need to develop sensitive, specific and faster tests as an aid in the early diagnosis of the primary tumor and its recurrence or malignant transformation in premalignant states.Aim: To estimate serum copper and zinc levels in Oral Submucous Fibrosisand oral squamous cell carcinoma patients. Methodology: Sera of OSCC (n = 10) and OSMF (n = 10) patients and of healthy controls was analysed for the estimation of Cu and Zn using atomic absorption spectrophotometry. Results: There was an increase in sera levels of Cu while those of Zn were decreased in both Oral Submucous fibrosis and Oral Squamous Cell Carcinoma patients as compared to the healthy controls. Conclusion: It could be concluded that there is an alteration of sera levels of these trace elements which can be helpful in early detection and management in OSMF and OSCC patients.

Key words: Oral Submucous fibrosis, oral squamous cell carcinoma, zinc, copper.

Corresponding Author
Rajeev Aggarwal, PG Student, Department of Oral and Maxillofacial Pathology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India. **E-Mail:** drrajeeev@yahoo.com

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Introduction

With anincrease in incidence of oral precancerous and cancerous lesions in Indian there is a necessity of in depth probing of various etiological as well as contributory factors for its early diagnosis and prognosis.[1]Dueto rapid economic development the adoption of a lifestyle with high risk habits further adds to the impact of cancer.[2] Among the various premalignant conditions of the oral cavity, Oral Submucous Fibrosisis of special concern not only due to its crippling condition that it produces in the patient but as the entire oral mucosa acquires malignant potential.[3]At present the confirmation of a lesion being precancerous and cancerous is done by histopathology. Recently, in the detection and diagnosis of oral cancer and precancerous lesions trace elements are receiving too much attention as they are found to be significantly altered in head and neck, lung and breast carcinomas. Trace elements have an important role in carcinogenesis.[1]Zinc and copper have been studied of the trace elements in patients with malignant disease and these elements in serum has been found to be reliable parameter as a diagnostic and prognostic index in case of craniofacial tumours.[4] Very few studies have been conducted to find out the role of different trace elements in oral precancer and cancer. Hence we present a comprehensive study to estimate levels of serum copper, zinc and Cu/Zn ratio in patients with Oral Submucous Fibrosisand Oral Squamous Cell Carcinoma in Northern India.

Material and Method

The study sample comprised thirty participants that were divided into four groups as:

- Group A: Patients with clinical and histopathologically diagnosed Oral Submucous Fibrosis, n = 10
- Group B: Patients with clinical and histopathologically diagnosed Oral Squamous Cell Carcinoma, n = 10

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- Group C: Healthy controls without any habit and without lesion (tobacco, betel nut, and alcohol consumption), $n = 5$
- Group D: Healthy controls with habit but without any lesion (tobacco and/or betel nut and/or alcohol consumption), $n = 5$

Methodology

A detailed oral examination of the patients was carried out using diagnostic instruments (mouth mirror and probe), and findings were recorded. A clinical diagnosis of Oral Submucous Fibrosis or the malignant ulcer was made based on the clinical appearance of the lesion, and a detailed case history was taken. Biopsy was taken from the site of the lesion. Biopsy tissue was processed and stained, and the diagnosis was histopathologically confirmed. 2ml of intravenous blood was drawn under aseptic precautions. The blood was allowed to clot and centrifuged at 1000 rpm for 15 min to separate serum. Serum was subjected to digestion using concentrated nitric acid and perchloric acid. For the determination of serum Cu, the sample was diluted with an equal volume of deionized water. For the determination of serum Zn, the sample was diluted in a ratio of 1:5 with deionized water. Estimation of serum Cu and Zn levels was done using AAS (Perkin Elmer, Shelton, CT 06484, USA Analyst 200/4000 spectrometer).

Stastical analysis

Data were entered into Microsoft Excel sheet and analyzed using Statistical Package for Social Sciences (Graph pad prism, Version 6.07) (SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) was used to compare the results of patients of Oral Submucous Fibrosis, Oral Squamous Cell Carcinomapatients, and the controls. Independent sample's *t*-test was used to compare the mean values between the two groups. Comparison of mean values between different groups was done using the Student's *t*-test using appropriate level of significance and degree of freedom.

Results

The mean serum copper levels in OSMF and OSCC was significantly increased as compared to healthy control with habit or without habit as seen in Table I. In [Table II], statistical analysis by ANOVA test showed a mean serum Zn level in OSMF patients of 80.40 ± 6.610 µg/dl and in OSCC patients of 59.63 ± 6.846 µg/dl which were significantly lower ($P < 0.0001$) as compared to the healthy controls in Group C, wherein a mean serum Zn level of 97.67 ± 4.865 µg/dl and Group D wherein a mean of 100.1 ± 4.559 µg/dl were found.As per [Table III], statistical analysis using ANOVA test showed mean serum Cu/Zn ratio in OSMF patients of 1.682 ± 0.1433 µg/dl and in OSCC patients of 2.323 ± 0.3225 µg/dl which were found to be significantly greater with a $P< 0.05$ as compared to the healthy controls in Group C, wherein a mean 1.106 ± 0.0678 µg/dl and group D, wherein a mean of 1.057 ± 0.0677 µg/dl were found. In [Table IV] the mean serum Cu/Zn levels in OSMF and OSCC were significantly increased as compared to the control groups.

Discussion

Oral cancer is ranked as eleventh most common type of cancer worldwide with over 130,000 reported deaths annually. Most commonly seen in south and south East Asian countries such as India, Taiwan, Bangladesh and Srilanka.[5]India has one of the highest incidence of oral cancer in the world. The development of cancer is a multistep process arising from pre-existing potentially malignant lesion.[2]There is also a high rate of malignant transformation of premalignant states like oral leukoplakia and Oral Submucous fibrosis.

In the present study, serum Cu levels were analyzed among Oral Submucous Fibrosis, Oral Squamous Cell Carcinoma, and the select control groups. Study results revealed that increase in mean serum Cu levels were observed in Oral Squamous Cell Carcinoma and Oral Submucous Fibrosis groups when compared to the control groups.

The results were similar to the study done by Haines *et al[6] in which* Cu levels were higher than the control group. KhannaS[7], Jayadeep A *et al[8].*, Haider SM *et al[9]*. And Shetty SR *et al.[10]*, also reported increased Cu levels in the sera of patients with oral premalignant and malignant lesions.Margalith *et al*.[11] suggested that role of Cu ions in biological damage is due to superoxide radicals or other reducing agents such as ascorbate, which eventually leads to damage to proteins, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA), that are not repairable by cellular mechanisms, thereby initiating the malignant process. Jayadeep A *et al[8]*, in their study, reported that the rise in serum Cu levels might be due to increased turnover of ceruloplasmin (a Cu carrying globulin with essential oxidase activity) in carcinoma patients.Following chewing, the uptake of Cu into the epithelial cells occurs probably by a nonenergy dependent diffusion where it is either bound to the proteins (mainly metallothioneins) or transferred across the basement membrane.The exact mechanism of Cu-induced mutagenesis is not fully understood. Cu-induced DNA damage has been reported, and there is evidence to suggest that Cu may bind to the protein product of p53, the major tumor suppressor gene, resulting in alteration of its conformation.12Trivedy CR *et al.13* have also reported Cu-induced mutagenesis through the p53 aberrations in Oral Submucous Fibrosiswhich might be critical in the progression of the potentially malignant lesion to Oral Squamous Cell Carcinoma.

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However, the findings of the present study contradict the findings of the study conducted by Varghese *et al*.14who found a significant reduction in serum Cu levels in Oral Submucous Fibrosispatients. In the present study, the mean serum Zn levels were analyzed among three groups. The study results revealed that there was decrease in mean serum Zn levels among group A and group B compared to the control groups (C, D). The results were in accordance with the various studies conducted by Varghese I *et al14* in OL, OSF, Oral Squamous Cell Carcinoma, Abdulla M *et al15* in head and neck cancers, Jha IN *et al* in oral cancers and Toke GB and Dhamne BK in head and neck cancers, in the past. Decreased sera levels of zinc have, also, been reported in patients suffering from gastrointestinal cancers, gynecological tumors, lymphomas, and breast and lung carcinomas. Altered sera zinc levels have, also, been correlated with decreased appetite in patients with advanced malignancies, especially, oral cancers.

Similar findings were found in the study conducted by VashisthaA *et al16* on patients with oral cancer (oral squamous cell carcinoma) and oral sub-mucous fibrosis (Oral Submucous Fibrosis) associated with habit of tobacco consumption in any form as compared to the healthy controls.

Shetty SR *et al*,10 also, found decrease in sera levels of zinc in patients with oral pre-cancers and cancers as compared to the controls. Kapil U *et al*, 17 observed that 53% of oral cancer patients in Jharkand had serum zinc deficiency and the deficiency was higher in females as compared to the males.

This could be because the malignant cells probably require more zinc which is taken up from the serum causing low levels of zinc in it. As there is negative interaction between copper and zinc, an increase in copper level may cause subsequent reduction in zinc level as well.[18]

In the present study, mean serum Cu/Zn ratio was analyzed amongst the three groups and the study results revealed that there was a significant ($p <$ 0.0001) increase in mean serum Cu/Zn ratio in groups A and B when compared to the controls (Groups C, D).

Similar findings were observed in earlier studies done by Jayadeep A *et al*,[8]Shetty SR *et al*[10], Varghese I *et al*,[14] Abdulla M *et al*,[15]Jha IN *et al*,[19] Toke GB [20] and Altered copper zinc ratio has, also, been observed in patients suffering from pancreatic cancers, gastric cancers, lymphomas, malignant lung tumors and breast cancers.

Thus, from the present study, it can be inferred that serum copper and Cu/Zn ratio was significantly higher in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma patients while simultaneously, there was a significant reduction in mean serum zinc levels when compared with the controls.

Thus, the alteration in serum copper and zinc and Cu/Zn ratio can be used as a potential biomarker in early detection of numerous oral pre-cancerous lesions and conditions and cancers as well as their malignant transformation and turning into frank cancers at an early enough stage.

Table I: Comparison of Serum copper level

Groups	n	Maximum value µg/dl	Minimum value µg/dl	Mean± SD
Group A (OSMF)	10	127	140	134.8 ± 4.197
Group B (OSCC)	10	126	144	136.8 ± 5.499
Group C (with habit & without lesion)	5	98	115	108.3 ± 5.080
Group D (Without habit, without lesion)	5	96	112	106.1 ±5.257

Table II: Comparison of Serum copper level

Groups	n	Maximum value µg/dl	Minimum value µg/dl	Mean ± SD
Group A (OSMF)	10	72	90	80.40 ± 6.610
Group B (OSCC)	10	49	73	59.63 ± 6.846
Group C (with habit & without lesion)	5	90	104	97.67 ± 4.865
Group D (Without habit, without lesion)	5	92	107	100.1 ± 4.559

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Table III: Comparison of mean copper/Zinc ratio

Groups	n	Maximum value (µg/dl)	Minimum value (µg/dl)	Mean ± SD
Group A (OSMF)	10	1.47	2.05	1.682 ± 0.1433
Group B (OSCC)	10	1.78	2.93	2.323 ± 0.3225
Group C (with habit & without lesion)	5	1.02	1.24	1.106 ± 0.0678
Group D (Without habit, without lesion)	5	0.93	1.17	1.057 ± 0.0677

Table IV: Comparison of Mean Serum Copper, Zinc and mean copper/Zinc ratio

Groups	Mean Cu level (µg/dl)	Mean Zn level(µg/dl)	Mean Cu/Zn ratio(µg/dl)
Group A (OSMF)	134.8 ± 4.197	80.40 ± 6.610	1.682± 0.1433
Group B (OSCC)	136.8 ±5.499	59.63 ± 6.846	2.323 ± 0.3225
Group C (with habit & without lesion)	108.3 ± 5.080	97.67 ± 4.865	1.106 ± 0.0678
Group D (Without habit, without lesion)	106.1 ±5.257	100.1 ± 4.559	1.057 ± 0.0677

Conclusion

- Determination of sera levels of Cu and Zn is simple as well as an inexpensive procedure and can be used as an adjunct screening tool for determining risk in patients with potentially malignant Oral Submucous Fibrosis and/or frank Oral Squamous Cell Carcinoma.
- The significant alteration in the levels of Cu and Zn in sera may be due to the cellular metabolic changes that occur during the pathogenesis of Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma. Thus, more studies need to be conducted on these trace elements.

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